TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

85

6 Budget Plan

Application For Research Grant

OK

Date: May 9, 1955

1. Name of Investigator: Sam Soroff, Ph.B.

2. Title: Research Associate

3. Institution & Address: The Institute for Cancer Research and Lankenau Hospital Research Institute
7701 Burholme
Fox Chase, Philadelphia 11, Pa.

4. Project or Subject: Chemical and Physical Studies on the Tissue Proteins Involved in Chemical Carcinogenesis.

1003541202

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Cellular Proteins Involved in Carcinogenesis--Certain azo dyes, when fed to rats or mice, produce hepatomas. These same dyes have been found to combine with certain proteins of the liver. So far, no other species, nor organs other than the liver, have been found to be similarly affected by these particular dyes.

Apparently, the dyes combine metabolically with a specific protein fraction of the liver, and the liver tumors produced by the dye contain neither the dye nor the protein fraction which in normal liver binds the dye.

The protein fraction involved thus appears as a specific target in the process of carcinogenesis. This concept is strongly supported by the observation of deficiencies of the same type of protein fraction in unrelated malignant tumors produced by different means in different organs of different species. In other words, while the carcinogenic action of aminoazo dyes seems limited to the livers of rats and mice, and thus is specific, the protein fraction which they attack may be of broad significance in the conversion of a normal cell into a malignant one.

We have isolated this presumably important protein fraction, in the expectation that chemical and biological study of the protein entities involved will shed light on the basic mechanisms by which exogenous agents produce cancer. If these cell proteins are representative of specific targets in carcinogenesis, they may likewise be involved in the presumptive carcinogenic action of certain fractions of tobacco smoke.

/s/ H. D. Putney (?)

Business Officer of the Institution

6. Budget Plan: TOBACCO INDUSTRY RESEARCH COMMITTEE
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Salaries	$10,710.00
Expendable Supplies	1,800.00
Applice Permanent Equipment	415.00
Overhead (6%)	1,068.00
Other (travel, service contract on analytical ultra-centrifuge)	430.00
Total	$14,423.00

Date May 9, 1955

7. Anticipated Duration of Work: The work is part of a long term scientific project operating on a yearly fiscal basis. The present application represents our request for support during the period October 1, 1955 to September 30, 1956.

8. Facilities and Staff Available:

(a) Facilities: One analytical--preparative Spinco ultracentrifuge; one Klett Tiselius apparatus; one M Perkin--Elmer Tiselius apparatus; two walk-in cold rooms; one dark room; three laboratory rooms; two air-conditioned animal rooms; two preparative macroelectrophoresis cells; one electrophoresis convection apparatus; high speed centrifuges, Beckman DU and DK spectrophotometers, etc.

(b) Staff: Our research unit consists of:

1. Principal Investigator: Sam Sorof, Ph.D. (University of Wisconsin, 1949).
2. Research Assistant (full time): Dorothy E. Vogt, M.S. (Purdue University, 1955).
3. Research Assistant (full time): Emily M. Young, B.S. (University of Vermont, 1950).
4. Laboratory Helper (half time): Arthur Nelson

In addition, at this Institute, specialists in many biological and chemical desciplines are available for consultations and possible assistance. The Institute maintains breeding colonies of rats and a number of highly inbred mouse strains, various ascites tumors, excellent library, stock rooms, well equipped machine and repair shops with trained specialists. #9 - Additional Requirements - NONE

10. Additional Information (Including relation of work to other projects and other sources of supply):

(a) General: The above outlined project represents the principal research effort of our unit. All work done by this group is directly involved in this undertaking.

The funds requested in this application represent the only provision for this project within the over-all Institutional budget, with the exception of the salary of the principal investigator.

(b) Scientific

A number of aminoazo dyes have been found to produce specifically primary cancer of the livers of rats and mice. All other tested species and organs have been shown to be almost completely resistant to the carcinogenic action of these compounds.

(Continued on added page)

Signature /s/ Sam Sorof
Director of Projects in Carcinogenesis

/s/ H. D. Putiny (?)
Business Officer of the Institution

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10. Additional Information (including relation of work to other projects and other sources of supply):

b) Scientific (continued)

Drs. James A. and Elizabeth C. Miller of the University of Wisconsin have shown (1,2) that unknown derivatives of these ingested azo dyes unite only with liver proteins of the above species only. Furthermore, the more potent the carcinogen fed, the faster the accumulation of these protein-bound azo dyes in the liver. This combination of carcinogen with liver protein is a very firm one which can only be broken by complete degradation of the liver proteins, and thus far has only been formed by the intact animal. In vitro attempts to duplicate this combination have resulted in very weak attachments between proteins and azo dyes which can be easily split with various organic solvents. The Millers have reported that about 55 per cent of the protein-bound dyes are associated with the "soluble" proteins, as isolated by the present aqueous cytochemical techniques. These investogators also demonstrated the important fact that the azo-dye-induced liver tumor lacks these protein-bound carcinogens. This lack appears to be the result of the absence of such binding proteins, per se, or an inability to protein-bind azo dyes, since free azo dyes, uncombined with proteins, are present in these liver tumors. The Millers hypothesized that the in vivo formed derivatives of these azo dyes combine with certain proteins (enzymes) which are necessary for the control of the growth, but not for the life of liver cells. These proteins are thereby inactivated. In addition, these proteins are autosynthetic, i.e., they control the mechanism of their own protein reproduction. Hence, with each generation of liver cells there is less of these active growth-controlling enzymes. Eventually, a liver cell appears with less than the critical amount of these proteins, and as a consequence a series of irreversible, genetic reactions occur which result in the formation of the liver tumor cell.

Our findings, begun at the University of Wisconsin in collaborations with Dr. Philip P. Cohen and Drs. James A. & Elizabeth C. Miller, and continued at the Lankenau Hospital Research Institute and Institute for Cancer Research, have substantiated and extended this "protein deletion hypothesis." We found that a close pair of small electrophoretic components (labeled h_1 and h_2), consisting of the relatively basic proteins among the soluble proteins of rat liver, contain the bulk of the soluble protein-carcinogen derivatives during azo dye preneoplasia (3). In contrast to the preneoplastic liver and liver surrounding azo-dye-induced tumors, this "h" fraction is almost absent in the tumors themselves and their distant metastases (4). The presence of protein-bound azo dyes in preneoplastic livers and liver surrounding azo-dye-induced tumors parallels the presence of "h" proteins therein; while the lack of "h" proteins in tumors parallels the absence of protein-bound azo dyes in these tumors. The reduction in size of the "h" component appears to be associated with neoplasia itself, rather than rate of growth, per se, of the

10. Additional Information Etc.

b) Scientific (continued)

tumors, since regenerating liver and liver of the fasted rat both closely resemble electrophoretically the liver of the normal stock rat (5). Interestingly, a variety of unrelated tumors (e.g., 2-acetylaminofluorene-induced liver tumor, a number of transplanted tumors, one human tumor thus far) electrophoretically exhibit a similar deficiency in the amount of the "h" proteins (4). In addition, this similarity exists among a number of tumors investigated by others (e.g., fibrosarcomas induced by methycholanthrene and benzpyrene (6).)

At the Lankenau Hospital Research Institute and Institute for Cancer Research, we have been extending our study of these "h" proteins. Using a new technique (7), we have isolated a major ultracentrifugal class of soluble liver proteins and found that the major share of the soluble protein-bound dyes is present therein (8). In addition, new methods and principles of electrophoretic fractionation have been developed (9,10) which have been applied to the isolation of the "h" proteins. Essentially by these techniques in unpublished studies, we have isolated the h_1 and h_2 proteins. Interestingly, both h_1 and h_2 have protein-bound dyes. This may be interpreted to fit the observed fact that both types of proteins are almost lacking in the azo-dye-induced tumor. Some physical and chemical properties of these proteins have been investigated. These findings are now being prepared for publication (11). We plan to extend our physical and chemical studies of these proteins obtained from rats fed control and various azo dyes of differing carcinogenicities in order to attempt to shed additional light on the mechanism of azo dye carcinogenesis, in particular, and the nature of the malignant transformation, in general. Thus, if these cell proteins are representative of specific targets in carcinogenesis, they may likewise be involved in the presumptive carcinogenic action of certain fractions of tobacco smoke.

References

(1) Miller, E. C. and Miller, J. A. Cancer Research, 7, 468 (1947).

(2) Miller, E. C., Miller, J. A., Sapp, R. W. and Weber, G. M. Cancer Research, 9, 336 (1949).

(3) Sorof, S., Cohen, P. P., Miller, E. C., Miller, J. A. Cancer Research, 11, 383 (1951).

(4) Sorof, S. and Cohen, P. P. Cancer Research, 11, 376 (1951).

References

(5) Sorof, S., Claus, B. and Cohen, P. P. Cancer Research, 11, 873 (1951).

(6) Barry, G. T. Cancer Research, 10, 694 (1950).

(7) Sorof, S. J. Am. Chem. Soc., 75, 5443 (1953).

(8) Sorof, S., Golder, R. H., Ott, M. G. Cancer Research, 14, 190 (1954).

(9) Sorof, S. and Ott, M. G. J. Am. Chem. Soc., 76, 4740 (1954).

(10) Sorof, S., Ott, M. G., Young, E. M. Arch. Biochem. Biophys., in press.

(11) Sorof, S., Ott, M. G., Young, E. M. to be published.